

COMPARATIVE STUDIES OF THE TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE.

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THE growing popularity of neoarsphenamine in the treatment of syphilis, due largely to the greater ease of its administration as compared with arsphenamine, inasmuch as the solutions do not require neutralization with alkali, and may be injected with a syringe in concentrated form, renders advisable a clear conception of the relative toxicity and therapeutic activity of these compounds. The purpose of this investigation was a comparative study of the toxicity of arsphenamine and neoarsphenamine prepared by various laboratories; comparative studies of the therapeutic activity of arsphenamine and neoarsphenamine based upon their influence upon experimental trypanosomiasis in rats, is given separately.¹

Numerous reports in literature by Castelli,² Hata and Hirano,³ Hoke and Rühl,⁴ Hoppe and Schreiber,⁵ Kersten,⁶ Kochmann,⁷ Marschalkó and Verzprém,⁸ Pearce and Brown⁹ and Willeox and Webster¹⁰ indicate that the highest tolerated doses of salvarsan for rabbits varies from 60 to 204 mg. per kilo, the general average being 80 to 100 mg. In a previous study reported by us¹¹ rabbits

¹ Schamberg, J. F., Kolmer, J. A., and Raiziss, G. W.: A Comparative Study of the Trypanocidal Activity of Arsphenamine and Neoarsphenamine, *Am. Jour. Med. Sc.*

² Ueber Neosalvarsan. Bestimmung der Toxizität und der heilenden Wirkung bei experimentellen Spirochätenkrankheiten, *Ztschr. f. Chemotherapie*, orig., 1912-1913, i, 321-352.

³ Quoted by Roth: *Saikin-Gaku-Zasshi*, 1910, No. 244, p. 321.

⁴ Experimentelle Untersuchungen über die Beeinflussung des Kreislaufes und der Atmung durch das Salvarsan, *Ztschr. f. Exper. Path. u. Therap.*, 1911, ix, 332-339.

⁵ Ueber die Behandlung der Syphilis und metasyphilitischen Erkrankungen mit dem neuen Ehrlich-Hatasehen Arsenpräparat, *Verhandl. deutsch. Kong. f. innere Med.*, 1910, xxvii, 243-253.

⁶ Ueber vergleichende Tierexperimente mit Salvarsan und Neosalvarsan, *Centralbl. f. Bakt.*, orig., 1912, lxx, 399-381.

⁷ Die Toxizität des Salvarsans bei intravenöser Einverleibung nach Versuchen an Hund und Kaninchen, *München. med. Wchnschr.*, 1912, lxx, 18-19.

⁸ Histologische und experimentelle Untersuchungen ueber den Salvarsantod, *Deutsch. med. Wchnschr.*, 1912, xxxviii, 1222-1225.

⁹ The Toxicity of Salvarsan and Neosalvarsan, *Jour. Pharmacol. and Exper. Therap.*, 1917, ix, 354-355.

¹⁰ The Toxicology of Salvarsan, *British Med. Jour.*, 1910, i, 473-478.

¹¹ Schamberg, J. F., Kolmer, J. A., and Raiziss, G. W.: Experimental and Clinical Studies of the Toxicity of Dioxydiaminoarsenobenzoldichlorhydrate, *Jour. Cutan. Dis.*, June, 1917.

were found to tolerate from 60 to 80 mg. of arsenobenzol per kilo of body weight over a period of several weeks; rats tolerated much larger amounts, ranging from 70 to 100 mg. per kilo. Roth¹² has reported that in experiments conducted in the Hygienic Laboratory rabbits were found to tolerate the various salvarsan preparations in intravenous doses of from 60 to 100 mg. per kilo of body weight for at least two weeks, although certain samples killed in doses of 60 mg. He has also corroborated our findings indicating that rats were more tolerant, amounts ranging from 60 to 135 mg. per kilo being tolerated for two weeks' period, the results depending somewhat upon whether the drugs were given in 1 or 2 per cent. solutions.

Neosalvarsan has been found much less toxic than salvarsan, the reports of Castelli,¹³ Kersten,¹⁴ Marschalkó,¹⁵ Pearce and Brown,¹⁶ Spiethoff¹⁷ and Roth,¹⁸ indicating that the tolerated dose for rabbits by intravenous injection is from 150 to 300 mg. per kilo of body weight.

Practical Value of Toxicity Tests. Insofar as toxicity tests with the lower animals and especially the white rats are concerned, it may be stated here that their value is limited to the detection of what may be called the "lethal toxicity" of arspenamine and neoarsphenamine; they do not exhibit the transient untoward effects observed in persons receiving intravenous injections of arspenamine and neoarsphenamine and designated as the "nitritoid crisis" or "arsphenamine reaction." The cause or causes of these reactions cannot be definitely stated at the present time; our own studies (2 and 3) have indicated that several factors may be implicated, including faulty technic in the preparation of solutions, individual susceptibility and finally the presence of an unidentified toxic substance designated as "X" in some lots of arspenamine and neoarsphenamine. Animal tests, especially in the smaller animals, fail to detect these causes of transient reactions following the administration of arspenamine and neoarsphenamine; compounds of both classes, although acceptable on the basis of "lethal toxicity tests", may still produce reactions when administered to persons. However, animal tests for "lethal toxicity" conducted by administering increasing amounts of drug by a uniform method, are of value as a means of establishing a criterion of purity and freedom from certain injurious substances and may be accepted for determining the relative lethal toxicity of arspenamine and neoarsphenamine per gram of body weight.

¹² An Experimental Investigation of the Toxicity of Certain Organic Arsenic Compounds, Bull. No. 113, Hygienic Laboratory, July, 1918, pp. 7-39.

¹³ Loc. cit.

¹⁴ Loc. cit.

¹⁵ Ueber Neosalvarsan, Deutsch. med. Wchnschr., 1932, xxxviii, 1585-1587.

¹⁶ Loc. cit.

¹⁷ Experimentelle und klinische Untersuchungen mit Salvarsan-Serumlösungen. Med. Klinik, 1914, x, 584-586.

¹⁸ Loc. cit.

The Technic of Toxicity Tests. As is now well known, many factors may modify the results of toxicity tests and especially with arspheamine administered intravenously, such as the degree of concentration of solution, the amount of alkali in the form of sodium hydroxid employed for neutralization and the rate of injection; for comparative tests the technic must be uniform and the Hygienic Laboratory has standardized the methods which may be briefly described in this place, inasmuch as the results of the studies employing rats as the test animals reported in this paper, were conducted according to these methods as follows:

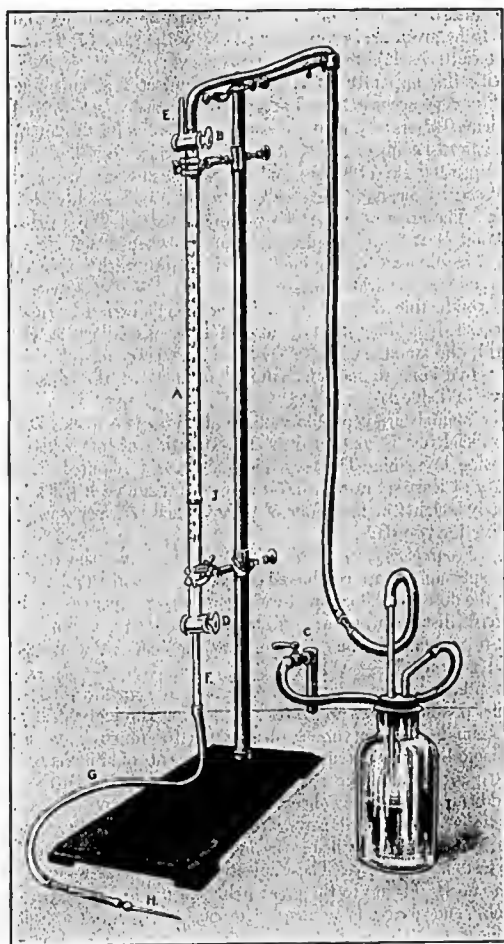
(a) *Arsphenamine Toxicity Tests.* Healthy white rats weighing for the most part between 100 and 150 gm. are employed; pregnant animals are excluded. All animals are kept under observation for at least ten to fourteen days before being used in these tests. Each animal is weighed prior to injection and the dose administered per body weight. All animals are fed late in the afternoon of the previous day in order that the weights may be taken and the injections made after a period of about eighteen hours' fasting, to render the dosage per body weight more accurate.

A 2 per cent. solution of the disodium salt of arspheamine is prepared by weighing out 0.5 gm. of powder and dissolving in 20.5 c.c. of warm, sterile, freshly distilled water; after complete solution has occurred, 4.5 c.c. of normal sodium hydroxide is added to convert the solution of acid base into a slightly alkaline solution of the disodium salt. Each solution is now filtered through sterile paper into a sterile vial and administered at once.

The injections are given in a saphenous vein exposed by a small incision; the gravity method is used for making the injections, employing the special apparatus designed by one of us (Kolmer), after the apparatus used by Dr. Lake in the Hygienic Laboratory. This apparatus is composed of a 2 c.c. burette divided into 0.01 c.c. (A) and fitted with a two-way cock at the upper end (B) for filling by means of suction by vacuum (C) and a water-tight cock at the lower end (D) for stopping the injection. The opening at (E) admits air to the pipette during the injection when the cock at (B) is turned after filling the pipette with solution, to cut off the vacuum. A long glass nozzle (F) is attached to the burette fitted with a short piece of best grade rubber tubing (G) carrying a window near the end, and a needle of No. 26 to No. 22 gauge (H).

Before injecting this solution the needle and rubber tubing are sterilized by boiling and the burette cleansed by copious flushing by means of the vacuum suction with sterile water followed by the solution to be injected, the water and solution of arspheamine collecting in the bottle (I) interposed between the vacuum and the burette. When solutions are changed the apparatus is cleansed between each in the same manner.

After the animal is bound upon the operating board the skin of the anterior surface of the thigh is cleansed with alcohol and a



small incision made over the vein, which is rendered prominent by pressure made in the inguinal region by an assistant. The vein

is grasped with fine forceps, due care being taken not to produce pain by grasping the nerve alongside of the vein, the needle is inserted and the cock (*D*) gradually opened to regulate the flow while the time in seconds is called off by an assistant, until the dose to be given previously marked off by the rider (*J*) fastened to the burette, is injected.

With this apparatus it is possible to inject a given amount of solution very accurately and at a given rate of flow set down by the Hygienic Laboratory at 0.5 c.c. per sixty seconds; the amount of solution to be injected is calculated according to the dose to be given per 100 gm. of weight and the rate of injection can be nicely regulated by means of the stop cock at (*D*) and timed with a stop clock. The apparatus is to be recommended for the intravenous injection of rats, guinea-pigs and rabbits, when a definite rate of flow is required in the conduct of toxicity tests with arspfenamine, tuberculin and various other drugs.

In conducting the toxicity tests the weight and sex of each rat is recorded, as likewise the dose of drug per kilogram of body weight, the amount of 2 per cent. solution carrying this dose and the actual time in seconds required for making the injection, as shown in Table I.

The animals are kept under observation for two weeks, although the official test is concluded at the expiration of forty-eight hours following injection. The Hygienic Laboratory requires the injection of at least six rats with a dose of 0.100 gm. each per kilogram of body weight; at least four of these animals (75 per cent.), must survive for two days.

(b) *Neoarsphenamine Toxicity Tests.* Tests for the toxicity of neoarsphenamine are conducted in exactly the same manner except that the doses per kilogram of weight are larger, 4 per cent. solutions in sterile distilled water are injected and the vein is tied after injection to prevent bleeding, inasmuch as the coagulation time of the blood is lowered.

In preparing the solutions we dissolve 0.8 gm. in 20 c.c. cold sterile, distilled water and filter the solution through sterile paper; the animals are kept under observation for fourteen days. The Hygienic Laboratory requires that 75 per cent. of animals receiving 0.200 gm. per kilogram shall survive for at least seven days.

Additional tests have been conducted by us by subcutaneous injections of mice and rats with solutions of arspfenamine and neoarsphenamine, this being the method of conducting these toxicity tests in Germany. These injections were made under the skin of the back. Owing to the great irritation produced by these compounds and particularly arspfenamine, ulcers were occasionally produced and the results were seldom as sharp and clear cut as those following intravenous injections; furthermore, the intravenous route is preferable by reason of the fact that both arspfenamine

and neoarsphenamine are commonly administered by this route in the human subject.

Scope of Investigation. In conducting this investigation arsphenamine and neoarsphenamine from six different laboratories designated by the numerals 1, 2, 3, 4, 5 and 6, were tested. Increasing amounts of these compounds were administered to rats by intravenous and subcutaneous injection, and to mice by subcutaneous injection. In each experiment the technic was identical in order to elicit the differences in toxicity of arsphenamine and neoarsphenamine due to the solutions of the drugs themselves.

Results. The results of toxicity tests conducted by intravenous injection of rats with arsphenamine and neoarsphenamine are shown in Tables I, II and III; Table I is a record of the results observed and Tables II and III summarize the results of these tests by stating the largest amounts of arsphenamine and neoarsphenamine tolerated over a period of seven days.

TABLE I.—TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE BY INTRAVENOUS INJECTION IN RATS.

No.	Wt., gms.	Sex.	Compound.	Dose per kilo.	Amount injected c.c.	Time of injection seconds.	Results in days.		
							At once.	2	3
1	125	M.*	Arsphenamine (Lab. No. 2)	0.08	0.50	60	—†	—	—
2	120	M.		0.08	0.48	57	—	—	—
3	95	F.		0.09	0.43	52	—	—	—
4	100	F.		0.09	0.45	54	—	—	—
5	80	M.		0.10	0.40	48	—	—	—
6	75	M.		0.10	0.38	45	—	—	—
7	85	M.		0.11	0.46	55	—	D.	—
8	95	M.		0.11	0.52	62	—	—	—
9	85	M.		0.12	0.51	61	—	D.	—
10	80	M.		0.12	0.48	57	—	—	—
11	115	M.	Neoarsphenamine (Lab. No. 2)	0.20	0.58	69	—	—	—
12	135	M.		0.20	0.68	82	—	—	—
13	125	M.		0.22	0.60	83	—	—	—
14	90	M.		0.22	0.50	57	—	—	—
15	105	M.		0.25	0.66	79	—	—	—
16	125	M.		0.25	0.78	94	—	—	—
17	85	M.		0.28	0.60	72	—	—	—
18	140	M.		0.28	0.98	118	—	—	—
19	115	M.		0.30	0.86	103	—	—	—
20	75	M.		0.30	0.56	67	—	D.	—

* M., male; F., female.

† Lived; D., died.

Tables IV and V represent the results observed with subcutaneous injections of solutions of arsphenamine and neoarsphenamine given to rats and mice; a summary of the results of tests of this kind is presented in Table VI.

TABLE II.—TOXICITY OF ARSPHENAMINE BY INTRAVENOUS INJECTION IN RATS.

Compound.	Highest dose per kilogram of weight tolerated for seven days.								Average.
	Exper. 1.	Exper. 2.	Exper. 3.	Exper. 4.	Exper. 5.	Exper. 6.	Exper. 7.	Exper. 8.	
Lab. No. 1	0.11 more than	0.10 more than	0.09	0.100
Lab. No. 2	0.12	0.12	0.10	0.10	0.10	0.10	0.10	0.10	0.105 +
Lab. No. 3	0.12	0.10	0.10	0.106
Lab. No. 4	0.12	0.10	0.10	0.11	0.105 +
Lab. No. 5	0.11	0.12	0.10	0.110
Lab. No. 6	0.10	—

TABLE III.—THE TOXICITY OF NEOARSPHENAMINE BY INTRAVENOUS INJECTION IN RATS.

Compound.	Highest dose per kilogram of weight tolerated for seven days.										Average.
	Exper. 1.	Exper. 2.	Exper. 3.	Exper. 4.	Exper. 5.	Exper. 6.	Exper. 7.	Exper. 8.	Exper. 9.	Exper. 10.	
Lab. No. I	0.28 more than	0.20 more than	0.18	0.25	more than	..	more than	..	0.228
Lab. No. 2	0.30	0.30	0.28	0.28	0.22	0.25	0.30	0.225	0.30	0.28	0.278 +
Lab. No. 3	0.28	0.22	0.25	0.250
Lab. No. 4	0.20	0.25	0.28	0.243
	more than
Lab. No. 5	0.25	0.25	0.28	0.30	0.270 +

TABLE IV.—TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE BY SUBCUTANEOUS INJECTION IN RATS.

No.	Wt., gms.	Sex.	Compound.	Dose per kilo. gms.	Amount of solu- tion injected, c.c.	Results in days.							
						At once.	1	2	3	4	5	6	7
1	95	F.	Arsphenamine (Lab. No. 2)	0.533	1.7	—	—	—	—	—	—	—	—
2	110	M.		0.533	2.0	—	D.	—	—	—	—	—	—
3	115	M.		0.400	1.57	—	—	—	—	—	—	—	—
4	100	M.		0.400	1.36	—	D.	—	—	—	—	—	—
5	120	M.		0.200	1.0	—	—	—	—	—	—	—	—
6	140	M.		0.200	1.0	—	—	—	—	—	—	—	—
7	160	M.		0.133	1.0	—	—	—	—	—	—	—	—
8	250	M.		0.133	1.1	—	—	—	—	—	—	—	—
9	105	M.		0.066	1.0	—	—	—	—	—	—	—	—
10	145	M.		0.060	1.0	—	—	—	—	—	—	—	—
11	115	M.	Neoarsphenamine (Lab. No. 2)	0.533	2.3	—	D.	—	—	—	—	—	—
12	150	M.		0.533	2.0	—	D.	—	—	—	—	—	—
13	00	M.		0.400	1.3	—	—	D.	—	—	—	—	—
14	115	M.		0.400	1.7	—	D.	—	—	—	—	—	—
15	140	M.		0.200	1.0	—	—	—	—	—	—	—	—
16	105	M.		0.200	0.5	—	—	—	—	—	—	—	—
17	00	F.		0.133	0.5	—	—	—	—	—	—	—	—
18	175	F.		0.133	0.5	—	—	—	—	—	—	—	—
19	210	M.		0.006	0.5	—	—	—	—	—	—	—	—
20	100	M.		0.006	0.5	—	—	—	—	—	—	—	—

TABLE V.—TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE BY SUBCUTANEOUS INJECTION IN MICE.

No.	Wt., gms.	Compound.	Dose per kilo. gms.	Amount of solution injected, c.c.	Results in days.							
					At once.	1	2	3	4	5	6	7
1	22	Arsphenamine (Lab. No. 2)	0.533	0.5	—	D.						
2	20		0.533	0.5	—	D.						
3	21		0.400	0.5	—	—	—	—	—	—	—	D.
4	19		0.400	0.5	—	D.						
5	19		0.200	0.5	—	—	—	—	—	—	—	
6	23		0.200	0.5	—	—	—	—	—	—	—	
7	22		0.133	0.5	—	—	—	—	—	—	—	
8	22		0.133	0.5	—	—	—	—	—	—	—	
9	17		0.066	0.5	—	—	—	—	—	—	—	
10	18		0.060	0.5	—	—	—	—	—	—	—	
11	22	Neearsphenamine (Lab. No. 2)	0.533	0.5	—	D.						
12	18		0.533	0.5	—	D.						
13	13		0.400	0.5	—	—	—	—	—	—	—	
14	23		0.400	0.5	—	—	—	—	—	—	—	
15	22		0.200	0.5	—	—	—	—	—	—	—	
16	17		0.200	0.5	—	—	—	—	—	—	—	
17	18		0.133	0.5	—	—	—	—	—	—	—	
18	10		0.133	0.5	—	—	—	—	—	—	—	
19	22		0.000	0.5	—	—	—	—	—	—	—	
20	18		0.000	0.5	—	—	—	—	—	—	—	

TABLE VI.—TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE BY SUBCUTANEOUS INJECTION IN MICE AND RATS.

Compound.	Animal.	Highest dose per kilogram tolerated for seven days.							Average.
		Exper. 1.	2.	3.	4.	5.	6.	7.	
Arsphenamine	Mouse	0.20	0.13	0.20	0.200	0.07	0.070	0.130	0.143
Neoarsphenamine	Mouse	0.20	0.40	0.20	0.330	0.33	0.400	0.200	0.280
Arsphenamine	Rat	0.20	0.40	0.40	0.200	0.20	0.400	0.533	0.342
Neoarsphenamine	Rat	0.20	0.20	0.20	0.133	0.20	0.133	..	0.177

A general summary of the results of this investigation is given in Table VII showing the highest, lowest and average dose of arspenamine and neoarsphenamine per kilogram of body weight tolerated by mice and rats by subcutaneous and intravenous injection.

TABLE VII.—SUMMARY SHOWING COMPARATIVE TOXICITY OF ARSPHENAMINE AND NEOARSPHENAMINE.

Compound.	Animal.	Route of injection.	Tolerated doses per kilo in seven days.		Average.
			Highest.	Lowest.	
Arsphenamine	Mouse	Subcutaneous	0.20	0.07	0.143
Neoarsphenamine	Mouse	Subcutaneous	0.40	0.20	0.286
Arsphenamine	Rat	Subcutaneous	0.40	0.20	0.342
Neoarsphenamine	Rat	Subcutaneous	0.20	0.13	0.177
Arsphenamine	Rat	Intravenous	0.12	0.09	0.105
Neoarsphenamine	Rat	Intravenous	0.30	0.18	0.254

Summary. These results may be summarized as follows:

1. Arsphenamine when injected intravenously in rats in the form of 2 per cent. solutions of the disodium salt in water after the method described, is generally borne in an amount averaging 0.105 gm. per kilogram of body weight corresponding to 7.35 gm. for a person weighing seventy kilograms or about one hundred and fifty pounds and representing an amount twelve times greater than the maximum dose (0.6 gm.) given at one time to an adult in the treatment of syphilis. The products of the different laboratories chosen at random for this study, were closely similar in toxicity; the range of tolerance over a period of seven days was from 0.090 to 0.120 gm. per kilogram of weight. Some lots of arsphenamine are tolerated by rats in larger amounts ranging to 0.150 gm. and even higher per kilogram of weight, but the average highest tolerated dose with arsphenamine dispensed at the time these studies were made appeared to be between 0.100 and 0.110 gm. per kilo. As previously stated, the Hygienic Laboratory requires that arsphenamine be borne by rats for two days in 0.100 gm. per kilogram of weight, and this appears to be a sufficiently high standard of purity.

2. Neoarsphenamine when injected intravenously into rats in a 4 per cent. solution in water is borne for seven days in doses varying from 0.180 to 0.300 gm. or more per kilogram of body weight; the average tolerance is about 0.254 gm. per kilogram of body weight which is equivalent to about 17.5 gm. for a person weighing seventy kilograms and about nineteen times more than the maximum dose (0.9 gm.) given in one injection in the treatment of syphilis. The Hygienic Laboratory requires that neoarsphenamine pass animal tests at 0.200 gm. per kilo of body weight over a period of seven days. Inasmuch as the toxicity of different lots of the compound varies considerably owing to the intricate chemical processes of manufacture, this would appear to be a fair index of purity for these compounds.

3. By intravenous injections in rats, neoarsphenamine is therefore about 2.4 times less toxic than arsphenamine:

$$\frac{\text{Average tolerated dose of neoarsphenamine, 0.254 gm. per kilo}}{\text{Average tolerated dose of arsphenamine, 0.105 gm. per kilo}} = 2.4$$

Taking 0.6 gm. arsphenamine and 0.9 gm. neoarsphenamine as the dose administered to adult persons in the treatment of syphilis, this dose of arsphenamine represents an amount twelve times less than the highest average tolerated dose for the rat and of neoarsphenamine nineteen times less than the highest average dose. If the results of these toxicity tests on rats can be applied to persons it is therefore evident that doses of neoarsphenamine greater than 0.9 gram may be given and yet remain within the same range of safety as the 0.6 gm. dose of arsphenamine.

4. Curiously arspfenamine is borne in larger doses than neoarsphenamine when injected into rats subcutaneously. Arspfenamine was tolerated in doses ranging from 0.200 to 0.533 gm. per kilogram of body weight with an average of 0.342 gm.; neoarsphenamine was tolerated in doses varying from 0.133 to 0.200 gm. per kilogram of weight with an average of but 0.177 gm.

5. When injected subcutaneously in mice arspfenamine was tolerated in amounts ranging from 0.070 to 0.200 gm. per kilogram of body weight averaging 0.143 gm.; neoarsphenamine was tolerated in doses ranging from 0.200 to 0.400 gm. averaging 0.286 gm. Castelli²⁰ found that while mice tolerated 0.143 gm. salvarsan and 0.250 gm. neosalvarsan when injected intravenously, the degree of tolerance was reversed when the compounds were injected subcutaneously, the tolerated doses being 0.250 gm. salvarsan and but 0.085 gm. neosalvarsan per kilogram of weight. Our results with a few experiments employing mice did not confirm these findings although with rats we found a reversal in tolerance similar to the results observed by him with mice.

6. Castelli also tested the toxicity of salvarsan and neosalvarsan for the hen, pigeon and rabbit, a summary of his results being given in Table VIII; many of our tests for the toxicity of arspfenamine made several years ago were conducted by injecting rabbits intravenously with a piston syringe, the total amount of fluid injected being 10 c.c., and given at a rapid rate into the ear vein. With this technic we found the toxicity of arspfenamine at this time for rabbits to be in the neighborhood of 0.080 gm. per kilogram of body weight indicating that the smaller animal (such as the rat) tolerates these arsenical compounds proportionate to weight better than the larger animal (rabbit). It is of interest to note that Castelli's results are similar, inasmuch as mice tolerated larger doses per kilogram of weight than rabbits.

TABLE VIII.—THE TOXICITY OF SALVARSAN AND NEOSALVARSAN AFTER CASTELLI.

Animal	Route of administration.	Dosis tolerata per kilogram.	
		Salvarsan.	Neosalvarsan.
Mouse	Intravenous	0.143	0.250
	Subcutaneous	0.250	0.085
Hen	Intravenous	0.080	0.060
	Intramuscular	0.250	0.010
Pigeon	Intravenous	0.080	0.120
	Intramuscular	0.090	0.040
Rabbit	Intravenous	0.100	0.200
	Subcutaneous	0.150	0.100

Conclusions. 1. Toxicity tests of arspfenamine and neoarsphenamine among the lower animals possess definite practical

²⁰ Loc cit.

value as a means of establishing standards of purity for these compounds.

2. These toxicity tests are best conducted by injecting solutions of the drugs intravenously inasmuch as this is the usual method of administration in the treatment of syphilis and the results are sharper than observed with subcutaneous injections.

3. Animal tests show "lethal toxicity" only, that is, the duration of life after the administration of given amounts per gram of body weight; they do not give rise to the transient untoward effects of arspenamine and neoarsphenamine ascribed to faults of technique in the preparation and injection of the solutions and the presence of an unidentified toxic substance designated as "X," which we believe may be present in the compounds themselves and produce the "nitritoid crisis."

4. The highest tolerated doses of arspenamine and neoarsphenamine administered by intravenous injection to healthy rats are about 0.105 and 0.254 gm. per kilogram of body weight respectively; neoarsphenamine is therefore about 2.4 times less toxic than arspenamine. Calculated upon the basis of seventy kilograms as the body weight of an average person, the highest tolerated dose of arspenamine may be placed at 7.35 gm. and of neoarsphenamine at 17.5 gm., providing the tissues of persons are approximately of the same susceptibility; comparative tests among rabbits, rats and mice in which the same amounts of drugs were given per gram of body weight indicate, however, that the larger and heavier animals are more susceptible and very probably human subjects cannot tolerate these substances in doses proportionate to body weight as established in animals.

5. By subcutaneous injection in mice neoarsphenamine was found to be half as toxic as arspenamine but when administered subcutaneously to rats, neoarsphenamine was found twice as toxic as arspenamine.

6. Insofar as the toxicity of arspenamine and neoarsphenamine, may be determined by intravenous injection of solutions in rats, the single dose of arspenamine commonly administered (0.6 gm.) may be said to be about twelve times less than the highest tolerated dose and the highest single dose of neoarsphenamine commonly injected (0.9 gm.) is about nineteen times less; from the standpoint of margin of safety larger amounts of neoarsphenamine may be given and maintain the same ratio between *dosis therapeutica* and *dosis tolerata*, as apparently exists with arspenamine.

A BRIEF EXPERIENCE WITH APPENDICOSTOMY AND CECOSTOMY FOR INTESTINAL STASIS IN EPILEPSY AND NEURASTHENIA.¹

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I HAVE had an opportunity in the last year or two to observe a small group of patients—two with neurasthenic symptoms and two with epilepsy—who had a considerable degree of intestinal stasis and in whom appendicostomy or cecostomy was done with subsequent washing out of the large intestine for a period of six to twenty-six months. The operation was done on the theory that delay in the passage of the contents of the colon and possible absorption of toxic material was responsible for some or all of these symptoms, and that the patient would be relieved by keeping the bowel well cleaned out.

The patients had all had a long, thorough course of medical treatment under the best conditions; some of them had stayed for a year or more at one of our best sanitariums, with little or no improvement of symptoms, and the operation was only done as a last resort. They were all patients seen in consultation and had been considerably encouraged by other physicians, who had seen them, to have the operation performed. Personally I did not have much enthusiasm for the method, but I was placed in a fortunate position to be able to follow the cases and to observe the results of the operation and subsequent treatment.

The literature of the subject is rather scanty and confusing. We are very likely to find immediate good results reported and the late results not given. Naturally the late results are chiefly interesting.

These cases, though few in number, are reported largely to stimulate discussion and bring out the experience of other men at this meeting. It seems worth while to have the facts in these cases even if the results are not brilliant.

Symptoms and Signs. The two epileptic cases gave a typical history of epileptic attacks of long duration, namely, fourteen years. The other two cases had pronounced chronic neurasthenic symptoms, headaches, insomnia, attacks of indigestion, anorexia, loss of weight, poor circulation, fatigue, mental apathy, bad taste in the mouth, abdominal gas, occasional tenderness, some staining of the skin, etc. They were all habitually constipated and had a palpable "juicy" cecum, and a roentgen-ray examination showed considerable delay in the passage of barium and food material

¹ Read at the Twenty-second Annual Meeting of the American Gastro-enterological Association, Atlantic City, June 10, 1919.